

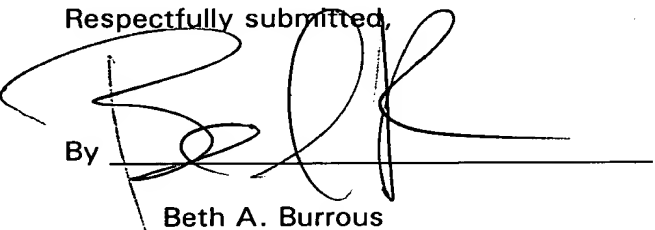
REMARKS

Applicants respectfully request that the foregoing amendments be made prior to examination of the present application.

Applicant believes that the present application is now in condition for allowance. Favorable consideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

By 

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

On page 8, delete the paragraph beginning on line 7 and ending on line 11 with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached.

Figures [1A and 1B] 1A - 1C. Human MKK1 nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2). Marked regions show the signal sequence, the SH2 and SH3 domains, and the catalytic domain.

On page 8, delete the paragraph beginning on line 18 and ending on line 22 with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached.

[Figures 3A and 3B] Figure 3. Human MKK3 nucleotide sequence (SEQ ID NO:5) and deduced amino acid sequence (SEQ ID No:6). Marked regions show the signal sequence, the SH2 and SH3 domains, and the catalytic domain.

On page 9, delete the paragraphs beginning on line 13 and ending on line 19, and replace these paragraphs with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached.

[Figure 9] Figures 9A and 9B. Shared amino acid sequence homology of MKK1 SEQ ID NO: 2 and csk SEQ ID NO: 7.

Figures [10A and 10B] 10A - 10C SEQ ID NOS 4, 8-10 respectively, in order of appearance. Shared amino acid sequence homology of MKK2 and atk/btk.

Figures [11A, 11B, 11C and 11D] Figures 11A - 11E SEQ ID NOS 6, 11-19, respectively, in order of appearance. Shared amino acid sequence homology of MKK3 and src tyrosine kinase family members.

On page 9, delete the paragraph beginning on line 27 and ending on line 29, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

[Figure 14] Figures 14A – 14D. [Figure 14] Figures 14A – 14D illustrate[s] the effect of tetradecanoyl phorbol acetate ("TPA") on either control cells or cells that express MKK-1.

On page 13, delete the paragraphs beginning on line 17 and ending on line 31, and replace these paragraphs with following in accordance with 37 CFR § 1.121. A marked up version showing changes made is attached.

The nucleotide and deduced amino acid sequence of human MKK1, MKK2, and MKK3 are shown in Figures 1A-[1B] 1C (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and [3A-3B] 3 (SEQ ID NOS 5-6), respectively. Figures 9 (SEQ ID NOS 2 and 7, respectively, in order of appearance), [10A-[10B] 10C (SEQ ID NOS 4, 8-10, respectively, in order of appearance) and 11A-[11D] 11E (SEQ ID NOS 6, 11-19, respectively, in order of appearance) show the shared sequence homology between MKKs and related tyrosine kinases.

5.1 The MKK Coding Sequences

The nucleotide coding sequence and deduced amino acid sequence of the human MKK1, MKK2, and MKK3 genes are depicted in Figures 1A-[1B] 1C (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and [3A-3B] 3 (SEQ ID NOS 5-6), respectively. In accordance with the invention, any nucleotide sequence which encodes the amino acid sequence of an MKK gene product can be used to generate recombinant molecules which direct the expression of an MKK.

On page 13 delete the paragraph beginning on line 32 and ending on page 14, line 8, and replace this paragraph with the following in accordance with 37 CFR §

1.121. A marked up version showing changes is attached:

In a specific embodiment described herein, the human MKK1, MKK2, and MKK3 genes were isolated by performing polymerase chain reactions (PCR) in combination with two degenerate oligonucleotide primer pools that were designed on the basis of highly conserved sequences within the kinase domain of receptor tyrosine kinases corresponding to the amino acid sequence HRDLAA (residues 350-355 of SEQ ID NO: 2) (sense primer) and [SDVWS/FY] SDVWSF/Y (SEQ ID NO:24) (antisense primer) (Hanks *et al.*, 1988). The MKK cDNAs were synthesized by reverse transcription of poly-A RNA from the human K-562 cell line, ATCC accession number CCL 243, or from the Meg 01 cell line, (Ogura *et al.*, Blood 66: 1384 (1985)).

On page 14, delete the paragraph beginning on line 9 and ending on line 14, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached.

The PCR fragments were used to screen a lambda gt11 library of human fetal brain. For each individual MKK, several overlapping clones were identified. The composite of the cDNA clones for MKK1, MKK2, and MKK3 are depicted in Figures 1A- [1B] 1C (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) , and [3A-3B] 3 (SEQ ID NOS 5-6), respectively.

On page 37, delete the paragraph beginning on line 18 and ending on page 38, line 7, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes made is attached.

cDNA was used in a polymerase chain reaction under standard conditions (*PCR Technology-Principles and Applications for DNA Amplifications*, H.E. Erlich, Ed., Stockton Press, New York 1989). Degenerate pools of primers corresponding to the amino acid sequence HRDLAA (residues 350-355 of SEQ ID NO:2) and SDVWSF/Y (SEQ ID NO:24) were prepared and used for the amplification:

5' oligo pool

H R D L A A
 5' GGAATTCC CAC AGN GAC TTN GCN GCN AG 3' (SEQ ID NO: 20)
 T C A T C A A C

3' oligo pool

F/Y S W V D S
 5' GGAATTCC GAA NGT CCA NAC GTC NGA 3' (SEQ ID NO: 21)
 ATG CA C C

Thirty-five PCR cycles were carried out using 8 µg (0.8 µg) of the pooled primers. (Annealing 55°C, 1 min; Extension 72°C, 2 min; Denaturation 94°C, 1 min). The reaction product was subjected to polyacrylamide gel electrophoresis. Fragments of the expected size (~210 bp) were isolated, digested with the restriction enzyme EcoRI, and subcloned into the pBluscript vector (Stratagene) using standard techniques (*Current Protocols in Molecular Biology*, eds. F.M. Ausubel *et al.*, John Wiley & Sons, New York, 1988).

On page 38, delete the paragraph beginning on line 18 and ending on page 39, line 5, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached.

The partial cDNA sequence of the new MKK1 TK, which was identified by PCR, was used to screen a λgt11 library from human fetal brain cDNA (Clontech)(complexity of 1×10^{10} recombinant phages). One million independent phage clones were plated and transferred to nitrocellulose filters following standard procedures (Sambrook, H.J., Molecular Cloning, Cold Spring Harbor Laboratory Press, USA, 1989). The filters were hybridized to the EcoRI/EcoRI fragment of clone MKK1, which had been radioactively labeled using 50µCi [α^{32} P]ATP and the random-primed DNA labeling kit (Boehringer Mannheim). The longest cDNA insert of ~3500 bp was digested with the restriction enzymes EcoRI/SacI to obtain a 5' end probe of 250 bp. This probe was used to rescreen the human fetal brain library and several overlapping clones were isolated. The composite of the cDNA clones of MKK1, MKK2 and MKK3 is shown in Figures 1A-[1B] 1C (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and [3A-3B] 3 (SEQ ID NOS 5-6), respectively. The 1.75 million independent phage clones of a human placenta library,

λ ZAP, were plated and screened with the 5' end probe (EcoRI/SacI) of the clone used above. Subcloning of positive bacteriophages clones into pBluscript vector was done by the *in vivo* excision protocol (Stratagene).

On page 39, delete the paragraph beginning on line 6 and ending on line 7, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached.

The composite cDNA sequence and the predicted amino acid sequence of MKK1, MKK2 and MKK3 are shown in Figures 1A-[1B] 1C (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and [3A-3B] 3 (SEQ ID NOS 5-6), respectively.

On page 40, delete the paragraph beginning 25 and ending on line 33, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached.

Antibodies recognizing MKK1 and MKK2 protein were made in rabbits using standard procedures. The anti-carboxy terminus MKK1 antibody was generated using the synthetic peptide GQDADGSTSPRSQEP (SEQ ID NO 22). The amino-terminus MKK1 Ab was generated using a GST-fusion proteins containing 78 amino acids coded by the SmaI to BG12 fragment of the MKK1 gene. The anti-carboxy terminus MKK2 Ab was made using a synthetic peptide corresponding to the sequence QQLLSIEPLREKDKH (SEQ ID NO 23).

In the Claims:

10. (Amended) An engineered host cell that contains the recombinant DNA vector of claim[s] 7[, 8, or 9].

15. (Amended) The isolated recombinant MKK1 of Claim 14 comprising the amino acid sequence depicted in Figures 1A [and 1B] - 1C (SEQ ID NOS 1-2).

17. (Amended) The isolated recombinant MKK2 of Claim 16 comprising the amino acid sequence depicted in Figures 2A and 2B (SEQ ID NOS 3-4).

19. (Amended) The isolated recombinant MKK3 of Claim 18 comprising the amino acid sequence depicted in [Figures 3A and 3B] Figure 3 (SEQ ID NOS 5-6).